#8550

FOOD COMPOSITION AND ADDITIVES

Determination of Crude Fat in Meat by Supercritical Fluid Extraction: Direct Method

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Abstract

Meat samples are prepared by passing meat through a food chopper, bowl cutter, or food processor, subsampling the meat, and mixing the meat with granular diatomaceous earth. No drying step is necessary. Supercritical CO_2 is then used to extract crude fat (which is defined as the components of meat that are extractable with petroleum ether, without digestion of the sample). Extracted material is deposited on glass wool contained in collection vials. After removal of any residual moisture from the extracts, percent crude fat is determined by weight gain of the collection vial. This method has been peer-verified by 3 laboratories, for a wide variety of raw and processed meat products containing 6–28% crude fat. Samples were prepared at the submitting laboratory. Ground samples were split into 4 portions, packed in Whirlpack bags, and immediately frozen. Frozen samples were sent by overnight mail to the peer laboratories. Samples were then thawed to room temperature, and percent fat was determined (in triplicate), without further processing of the samples. Analysis of the samples was completed within 1 week of sample preparation. On the basis of this study, it can be estimated that all repeatability and reproducibility values are <3.0. Mean accuracy of the direct gravimetric supercritical fluid extraction method for meat samples ranged from +0.22 to −1.41 when the method was compared with AOAC Method **960.39**. Interferences are unlikely but would include any nonfat substance that is added to (processed) meat, is soluble in nonpolar solvents, and is present in a quantity that would alter results. This method is expected to perform equally well for all meats with fat content within the stated range of applicability.

1 Summary of Results of Verification Study

Results obtained by the submitting and peer laboratories are shown in Table 1 and summarized in Table 2.

1.1 Matrixes

A total of 9 meat matrix types were studied, 3 raw and 6 cooked: raw ground beef, raw pork sausage, raw veal sausage, smoked ham, kielbasa, braunschweiger, sweet Italian sausage, smoked sausage, and bologna.

1.2 Number of Samples

The fat content in 2 different samples of smoked ham, 3 different samples of kielbasa, and one sample each of the remaining 7 matrix types was determined. The total number of samples was 12.

1.3 Accuracy and Recoveries

The mean accuracy of the direct gravimetric supercritical fluid extraction (SFE) method for meat samples ranged from +0.22 to −1.41 when the method was compared with AOAC Method **960.39** (1).

1.4 Precision—Repeatability and Reproducibility

Repeatability values for the direct gravimetric SFE method for meat samples ranged from 0.22 to 2.88. Reproducibility values ranged from 0.53 to 2.88.

1.5 Ruggedness

During development, the method was evaluated for ruggedness. The critical parameters were identified as follows:

(**1.5.1**) *Extraction time.*—Significant reductions in accuracy and repeatability were noted for some samples when ex-

traction time was shortened from 45 to 30 min. Excessive time did not have a detrimental effect on accuracy or precision. Extraction time should be held at 45 min for all samples.

(1.5.2) *Sample size.*—A significant reduction in extraction efficiency and incomplete extraction were noted when the total amount of moisture in the sample was >1.0 g. Precision was reduced for small sample sizes with low fat content. For these reasons, the appropriate sample size is 1.0–1.5 g. However, if the moisture content is >70%, the maximum sample size is 1.0 g.

(1.5.3) *Sample preparation.*—A significant reduction in precision was noted when samples were improperly ground or improperly subsampled.

(1.5.4) *Extraction temperature.*—It was found that lower temperatures often gave incomplete extraction and low results. Temperatures that were too high caused degradation of

Table 1. Raw collaborative data

Sample	Lab	Result 1	Result 2	Result 3
Raw ground beef	Peer 2	23.71	23.85	22.97
	Peer 1	22.90	22.20	23.53
	Submitting	22.30	22.30	NR[a]
Raw pork sausage	Peer 2	27.16	26.43	26.48
	Peer 1	27.22	28.28	28.26
	Submitting	26.00	26.30	NR
Smoked ham #1	Peer 2	11.09	11.26	11.16
	Peer 1	11.49	11.58	11.43
	Submitting	12.10	10.70	NR
Kielbasa #1	Peer 2	13.21	12.26	12.73
	Peer 1	13.01	12.80	12.40
	Submitting	12.00	12.00	NR
Braunschweiger	Peer 2	5.77	5.76	5.61
	Peer 1	5.93	5.98	5.74
	Submitting	5.69	5.26	NR
Sweet Italian sausage	Peer 2	13.85	13.84	13.33
	Peer 1	13.79	13.67	14.13
	Submitting	15.00	NR	NR
Smoked sausage	Peer 2	18.08	17.61	17.78
	Peer 1	18.18	18.47	18.48
	Submitting	18.00	18.30	NR
Raw veal sausage	Peer 2	19.47	19.72	19.90
	Peer 1	19.82	19.76	20.30
	Submitting	18.00	19.40	NR
Bologna	Peer 2	12.94	12.83	12.81
	Peer 1	13.21	13.35	13.41
	Submitting	12.40	12.90	NR
Kielbasa #2	Peer 2	15.65	17.06	18.78
	Peer 1	15.89	16.23	16.74
	Submitting	16.10	15.90	NR
Kielbasa #3	Peer 2	18.67	19.99	20.86
	Peer 1	20.68	20.11	21.08
	Submitting	20.00	20.60	NR
Smoked ham #2	Peer 2	12.25	12.19	12.29
	Peer 1	12.62	12.51	12.55
	Submitting	12.20	12.40	NR

[a] NR = not reported.

Table 2. Summary of collaborative results[a]

Lab	Sample	SFE avg.	$\bar{x}$	s_r	s_R	RSD_r	RSD_R	r	R	**960.39**	Accuracy
Peer 1	4192	22.88									
Peer 2	4192	23.51	22.90	0.52	0.71	2.25	3.11	1.45	1.99	23.77	−0.87
Submitting	4192	22.30									
Peer 1	4227	27.89									
Peer 2	4227	26.69	26.92	0.47	0.98	1.75	3.62	1.32	2.73	28.14	−1.22
Submitting	4227	26.30									
Peer 1	4229	11.50									
Peer 2	4229	11.17	11.36	0.45	0.45	3.95	3.95	1.26	1.26	11.34	0.02
Submitting	4229	10.70									
Peer 1	4234	12.75									
Peer 2	4234	12.73	12.49	0.36	0.48	2.87	3.87	1	1.35	12.54	−0.05
Submitting	4234	12.00									
Peer 1	4235	5.88									
Peer 2	4235	5.71	5.69	0.17	0.24	2.95	4.14	0.47	0.66	5.94	−0.25
Submitting	4235	5.26									
Peer 1	4236	13.86									
Peer 2	4236	13.67	14.18	0.27	0.6	1.90	4.2	0.75	1.67	14.3	−0.12
Submitting	4236	15.00									
Peer 1	4239	18.38									
Peer 2	4239	17.82	18.12	0.21	0.34	1.15	1.87	0.58	0.95	18.23	−0.11
Submitting	4239	18.30									
Peer 1	4240	19.96									
Peer 2	4240	19.69	19.45	0.50	0.73	2.57	3.77	1.4	2.05	20.86	−1.41
Submitting	4240	19.40									
Peer 1	4242	13.32									
Peer 2	4242	12.86	12.94	0.18	0.37	1.36	2.85	0.49	1.03	13.15	−0.21
Submitting	4242	12.90									
Peer 1	4244	16.29									
Peer 2	4244	17.17	16.48	1.03	1.03	6.25	6.25	2.88	2.88	16.26	0.22
Submitting	4244	15.90									
Peer 1	4282	20.63									
Peer 2	4282	19.84	20.25	0.79	0.79	3.88	3.88	2.2	2.2	20.16	0.09
Submitting	4282	20.60									
Peer 1	4287	12.56									
Peer 2	4287	12.24	12.37	0.08	0.19	0.64	1.52	0.22	0.53	12.51	−0.14
Submitting	4287	12.40									

[a] $\bar{x}$ = Overall mean (n = 8); r = repeatability value; s_r = repeatability standard deviation; R = reproducibility value; s_R = reproducibility standard deviation; accuracy = Method **960.39** − x; RSD_r = repeatability relative standard deviation; RSD_R = reproducibility standard deviation.

the matrix and excessive maintenance problems. The extraction temperature should be between 95 and 105°C.

2 Safety Precautions

Normal care should be exercised in handling the glass wool, diatomaceous earth, hot thimbles, and high-pressure gases used in this method.

3 Scope

The method was specifically tested for raw, cooked, and processed meat products. The tested range was 5–28% crude fat, in high-moisture meat samples. If sample size is <1.3 g, then natural moisture content is irrelevant, provided that moisture is <70%.

4 Reference

(1) *Official Methods of Analysis* (1995) 16th Ed., AOAC INTERNATIONAL, Gaithersburg, MD

5 Principle

Ground samples are weighed and dispersed on granular diatomaceous earth, which absorbs the moisture and gives the sample a large surface area. Prepared samples are transferred to high-pressure extraction thimbles, which position the sample between 2 fritted metal disks. The SFE instrument draws liquid CO_2 from a "dip-tube" tank into a refrigerated pump head, and the extraction solvent is compressed to 62.035×10^6 Pa (9000 psi). The compressed CO_2 exits the pump, is preheated, and passes downward through the heated thimbles (100°C), removing fat from the samples. Extracted material flows into the collection system, where the CO_2 is depressurized through heated variable-flow restrictors, and the analyte is quantitatively collected on glass wool contained in preweighed, removable collection vials. CO_2 passing through the collection system evaporates and exits through flow meters, giving a feedback signal for flow control. Residual extracted moisture is removed from the collection vials by using a microwave oven, and the vials are weighed again. Percent fat is a direct determination, based on the weight gain in the collection vials.

6 Reagents and Supplies

(**6.1**) *Granular diatomaceous earth.*—LECO-dry (P/N 502-327); Leco Corp., St. Joseph, MI), or equivalent. Description of material: flux calcined filter aid (manufactured from plankton marine diatomite) with median particle size of 55.0 μm, as measured by granulometer.

(**6.2**) *Glass wool.*—Borosilicate glass, 0.0051–0.0076 mm diam. fibers (P/N 501-081; Leco Corp., or equivalent).

(**6.3**) *Carbon dioxide (CO_2).*—Industrial (welding) grade, with siphon (dip) tube.

(**6.4**) *Laboratory wipes.*—Kim Wipes EX-L, 4.5 × 8.5 in. (Kimberly-Clark, Roswell, GA), or equivalent.

7 Apparatus

(**7.1**) *SFE system.*—TFE2000 fat determinator (Leco Corp.), or equivalent.

(**7.1.1**) *Minimum required specifications/configuration for equivalent apparatus.*—Sample thimbles, 10 mL internal volume; pressure capability, 62.035×10^6 Pa (9000 psi); thimble temperature capability, 100°C; heated variable-flow restrictors, capable of maintaining temperature of expanding CO_2 at a minimum of 30°C; collection vials, removable, and capable of containing a minimum of 1.2 g loosely packed glass wool, with restrictor outlet that deposits fat directly and deeply into glass wool; balance, capable of accurate weights, to the nearest 0.1 mg; and microwave oven, household type.

(**7.1.2**) *Operating conditions.*—CO_2 pressure, 62.035×10^6 Pa (9000 psi); thimble temperature, 100°C; CO_2 flow rate, 2.26 g/min (1.3 L/min, expanded gas at 35°C); heated variable restictor temperature, Leco systems = 100°C and other systems = appropriate temperature to maintain collection vial at 30–40°C during extraction; and extraction time (dynamic), 45 min. (The temperature-independent flow value of 2.26 g/min is given to allow conversion to the various flow measurement techniques that might be used in equivalent systems. Because of the temperature-dependent density of both gaseous and liquid CO_2, large errors can be introduced by failing to consider the temperature of the flow measurement. The manufacturer of an equivalent system should be consulted to determine the correct flow setting, based on the specific system of measurement.)

(**7.1.3**) *Required performance specifications.*—Pressure accuracy, ± 5% of set value; temperature accuracy (thimbles and restrictors), ± 5% of set value; and flow accuracy, ± 20% of set value, maintained throughout extraction.

(**7.1.4**) *Acceptance tests.*—Observation of stable and correct flow for each extraction channel, as outlined in required performance specifications; >99.0% recovery of 0.4 g refined soybean oil, spiked on granular diatomaceous earth; and after extraction of blank, final weight of vial should be within ± 2 mg of initial weight of vial.

8 Sampling

Use conventional, established sampling methods, as appropriate for matrix type.

9 Sample Preparation

To prevent water loss during preparation and subsequent handling, do not use small samples. Keep ground material in glass or similar containers with air- and water-tight covers.

9.1 Fresh Meats, Dried Meats, Cured Meats, and Smoked Meats

(**9.1.1**) *Food chopper.*—Separate as completely as possible from any bone; pass rapidly 3 times through food chopper with plate opening of ≤ 1/8 in. (3 mm), mixing thoroughly after each grinding; and begin all determinations promptly. If any delay occurs, chill sample to inhibit decomposition.

(**9.1.2**) *Bowl cutter.*—Alternatively, use a bowl cutter for sample preparation (benchtop model, ½ HP; 14 in. bowl, 22 rpm; two 3.5 in. knives, 1725 rpm; Model 84145, Hobart Corp., Troy, OH; or equivalent). Chill all cutter parts before preparation of each sample.

(**9.1.3**) *Food processor, AOAC Official First Action 1990.*—Benchtop model, 110/120 V, 60 Hz, 1 hp, 7.5 A, 1725 rpm, fan-cooled motor, 4 qt bowl; Model R4Y (Robot Coupe USA, Inc., Jackson, MS), or equivalent. (*Caution:* Do not remove cutter bowl lid or cutter bowl from base until motor has come to a full stop. Do not put hand, finger, or any object into bowl while motor is running. Unplug appliance before servicing or cleaning.)

Precut sample, up to 2 lb, to a maximum of ≤2 in., and transfer to bowl for processing. Include any separated liquid. Process 30 s, and then wipe down inner side wall and bottom of bowl with spatula (use household plastic or rubber spatula with approximately 2 × 4 in. straight-edge blade); transfer gathered material to body of sample. Continue processing another 30 s, and wipe down as before. Repeat sequence to give a total of 2 min processing and 3 wipe downs.

Take particular care with certain meat types such as ground beef to ensure uniform distribution of fat and connective tissue. At each wipe-down interval, reincorporate fat and tissue into sample by using spatula to remove fat from inside surfaces of bowl and connective tissue from around blades. If sample consolidates as a ball above the blades, interrupt processing and press sample to bottom of bowl with spatula before continuing.

Ref.: *J. AOAC Int.* **72**, 777(1989)

9.2 Canned Meats

Pass entire contents of can through food chopper, bowl cutter, or food processor, as in section 9.1.

9.3 Sausages

Remove from casings and pass through food chopper, bowl cutter, or food processor, as in section 9.1.

Chilled samples can be stored for approximately 1 week. *Note*: Additional sample preparation is required before sample is placed in extraction thimble, *see* section 11.2.

10 Controls Preparation

10.1 Blanks

A ¼ Kim Wipe plug is packed into bottom of thimble. Thimbles are then filled with granular diatomaceous earth.

10.2 Oil Spikes

A ¼ Kim Wipe plug is packed into the bottom of a thimble. The thimble is then filled to within 2 cm of the top with granular diatomaceous earth. The thimble is placed on a balance, and the balance is tared. Refined soybean oil is dripped from a pipet directly onto the center of the diatomaceous earth until the balance reads approximately 0.4 g. The weight of the oil is recorded to the nearest 0.1 mg.

11 Procedure

11.1 Preparation of Collection Vial

Cut 1.3–1.5 g glass wool from end of glass wool rope. Pull the compact section of glass wool apart so that the material is loosened considerably. Pack the loosened glass wool into the collection vial with a clean spatula, a little at a time. (The goal is to have random, not vertical orientation of the wool strands.) Tare the empty balance pan, and enter initial vial weights into the instrument (or record initial vial weights for manual calculation).

11.2 Preparation of Sample/Thimble

This procedure must be performed within 4 h of the start of the extraction. Place 2.2 g diatomaceous earth into a 50 mL beaker. Place beaker plus diatomaceous earth on balance and tare the weight. Using one spatula to hold the sample above the beaker, and another to scrape the sample from the first spatula, drop 1.0–1.5 g ground sample onto the diatomaceous earth. Enter sample weight into the instrument (or record sample weight for manual calculation). Remove beaker from the balance and thoroughly mix sample with diatomaceous earth, using a clean spatula. Prepared sample should not be sticky or adhere to the sides of the beaker (sample size must be reduced, if this is the case). Install a lower end-cap assembly on an extraction thimble. Pack ¼ of a Kim Wipe into the bottom of the thimble by folding it once, and pushing/packing it into the thimble with a clean spatula. Transfer the prepared sample into the thimble, using a funnel. Install the upper end-cap assembly on the extraction thimble.

11.3 Sample Extraction

Set up (or recall and activate) the instrument operating conditions previously specified. Install the prepared collection vials and extraction thimbles in the SFE instrument, according to the manufacturer's instructions. Start extraction (extraction thimbles should not be allowed to heat to extraction temperature, without pressurization). After extraction is completed and the system is depressurized, remove the collection vials and thimbles from the instrument.

11.4 Post-Extraction Manipulations

To remove residual moisture from the extracts, microwave the collection vials for 2 min at 1000 W setting of household microwave oven (high setting); let cool for 15 min. The wattage rating of the microwave oven is usually found on the serial

number sticker, or serial number plate. Time may be extended to 3–4 min for 750 W microwave ovens.

Tare the empty balance pan, and enter the final weight of each collection vial into the instrument (or record final vial weight for manual calculation).

11.5 Controls (Oil Spikes and Blanks)

Both oil spikes and blanks should be used periodically to verify proper instrument operation. Furthermore, they can also be used as a diagnostic tool to evaluate instrument equivalency and proper operator technique. This method should not be used until satisfactory results for oil spikes and blanks (as outlined in section 7) have been obtained.

Extractions of oil spikes and blanks should be used to determine instrument equivalency; to verify operator technique and expertise; and to verify correct instrument operation, at least weekly.

12 Calculations

% Crude fat = (final vial weight – initial vial weight) (100)/sample weight

13 Test Results Report

On the basis of this study, it can be estimated that all repeatability and reproducibility values are <3.0. Mean accuracy of the direct gravimetric SFE method for meat samples ranged from +0.22 to –1.41 when the method was compared with AOAC Method **960.39**. Collaborative results are listed in Tables 1 and 2. Interferences are unlikely but would include any nonfat substance that is added to (processed) meat, is soluble in nonpolar solvents, and is present in a quantity that would alter results. This method is expected to perform equally well for all meats with fat content within the stated range of applicability.

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